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Allosteric Coupling and Thermal Activation in TRP Channels
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Thermo-TRP can be directly gated by low or high temperature, doing so with outstanding temperature sensitivity and giving rise to the molecular basis for temperature sensing in animals. The mechanism of temperature sensitivity in these channels is associated with large changes in enthalpy (DHo) and entropy (DSo) upon channel gating. The magnitude, sign, and temperature dependence of DHo and DSo, the last given by an associated change in heat capacity (DCp), can determine a channel's temperature sensitivity and whether it is activated by cooling, heating, or both, if DCp makes an important contribution. Thermo TRP can also be activated by several chemical stimuli and show modest voltage-sensitivity. These channels have been shown to be allosteric proteins and several of their gating behaviors can be explained by assuming coupling between allosterically coupled gating modules and a pore module. We show that in the presence of such allosteric gating mechanism, other parameters, apart from DHo and DSo, which include the gating equilibrium constant, the strength- and temperature dependence of the coupling between gating and the temperature-sensitive transitions, as well as the enthalpy/entropy ratio associated with each transition, can strongly determine the temperature-dependent activity of a particular channel, giving rise to cold or hot activated channels operating with the same temperature sensor but inverted coupling. Also of interest we show that allosterically gated thermo-TRPs can respond to both cooling and heating in a DCp-independent manner.
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Toward the Mechanism of Capsaicin Binding to TRPV1 in a Lipid Bilayer via Atomistic Simulation
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Many lipophilic small molecule ligands (drugs and natural compounds) interact with membrane proteins, but often experimental approaches to address these interactions prove costly and time-consuming at best, intractable at worst. Studies of these ligands using molecular dynamics (MD) simulations in an explicit lipid bilayer system can aid our understanding of their possible mechanisms of action with the target membrane protein. Capsaicin, the active ingredient of chilli, and related vanilloid ligands specifically modify the activity of the TRPV1 heat-sensitive ion channel and are thought to interact with the transmembrane region of the protein. However, many details of this interaction such as the orientation of capsaicin and the involvement of other domains of the channel in ligand binding remain unknown. In this study we employ MD simulations to define the interactions of capsaicin with a lipid bilayer via equilibrium simulations, PMF calculations, and simulations with a homology model of rat TRPV1. Our simulations show that capsaicin spontaneously partitions into phospholipid bilayers and preferentially localizes at the water/bilayer interface. They also suggest the significance of capsaicin flip-flop from one side of the bilayer to the other. These and related results are discussed in the context of understanding the significance of the lipid bilayer environment to the orientation and kinetics of the interaction of capsaicin with TRPV1.

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Biophysical Characterization of the TRPM8 Voltage-Sensing Domain
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Transient receptor potential melastatin 8 (TRPM8) is an outwardly rectifying, nonselective cation channel and has been shown to be activated (gated) in a polymodal manner by voltage, chemical ligands, cold temperatures, lipids, and modulatory membrane proteins. Channel expression is most closely associated with sensory neurons, though it is expressed in many types of tissues. Since its discovery, much attention has been paid to TRPM8 because of its role in human health and disease. The most well-known function of the channel is in cold sensation and related cold-induced pain. However, the initial discovery of TRPM8 came about through its identification as an oncogene that is up-regulated in prostate, breast, colon, lung, and skin cancers. TRPM8 is also significant as a pain receptor and is considered a hopeful target for analgesic development. At the heart of unlocking the widespread therapeutic potential of TRPM8 rests on understanding how to open and close, or gate the channel. The voltage-sensing domain (VSD) of TRPM8 is key to gating and has been shown to form a nexus where at least chemical ligands and voltage are integrated to cause channel gating. Here we present the conditions used to express and purify the folded TRPM8 VSD, show initial solution NMR-based structural characterization, as well as far UV circular dichroism and differential scanning calorimetry biophysical studies.

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Crystal Structure of the N-Terminal Ankyrin Repeat Domain of TRPV3 Reveals Unique Conformation of Finger 3 Loop Critical for Channel Function
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Like other TRP channels, TRPV3 is a multimodal cation channel, and can be activated by stimuli including natural compounds such as camphor, thymol, endogenous ligands FPP, NO and synthetic small molecular 2-APB. In all six members of TRPV channel subfamily, there is an ankyrin repeat domain (ARD) in their intracellular N-termini. Ankyrin (ANK) repeat, a common motif with typically 33 residues in each repeat, is primarily involved in protein-protein interactions. Despite the sequence similarity among the ARDs of TRPV channels, the structure of TRPV3 ARD, however, remains unknown. In this study, we report the crystal structure of TRPV3-ARD solved at 1.95 Å resolution, which reveals six-ankyrin repeats. While overall structure of TRPV3-ARD is similar to ARDs from other members of TRPV subfamily; it, however, features a noticeable finger 3 loop that bends over and is stabilized by a network of hydrogen bonds and hydrophobic packing, instead of being flexible as seen in known TRPV-ARD structures. Electrophysiological recordings demonstrated that mutating key residues of finger 3 altered the channel activities and pharmacology. Taken all together, our findings show that TRPV3-ARD with characteristic finger 3 loop likely plays an important role in channel function and pharmacology.

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A Structural Framework for the Polymodal Pain Sensor TRPV1
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TRPV1 is an important polymodal cellular sensor for heat, capsaicin, and other noxious stimuli. How the channel is activated by diverse physical and chemical stimuli remains largely unknown. Structural information is critical for mechanistic investigation but is currently lacking for TRPV1 and its homologs. In order to provide a structural framework for the study of TRPV1 gating mechanism, we have first modeled the transmembrane region in both the closed and open states using the Rosetta method. Reliability of predicted structural models is supported by results from a combination of mutagenesis, fluorescence imaging and patch-clamp recording tests. We found that while the overall predicted structural architectures resemble those of other six-transmembrane tetrameric cation channels, there are a number of interesting unique structural features that may contribute to capsaicin sensitivity, coupling of conformational changes in the turret and other extracellular structures to the pore, as well as pore dilation upon activation. Extending these modeling efforts to the intracellular regions further indicated potential structural elements that may mediate subunit assembly and modulation by intracellular factors. Therefore, our model has setup a framework for further investigation of the molecular events that lead to TRPV1 activation.

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Conformational Plasticity of TRPV1 Ankyrin Repeat Domain in Complex with Cysteine Reactive Agonist Allicin
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Chemosensation is a signal transduction process in which exogenous compounds interact with receptors and give a taste, odor or other sensation. The TRPV1 ion channel is one of the most important chemoreceptors associated with pain. This channel is activated by pungent compounds like capsaicin (Chili peppers), low pH, high temperature, allicin (Garlic) and others. Allicin (diallylthiosulfinate) is non polar compound that reacts with cysteines forming an allylcystine.